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CITRUS RESEARCH CONFERENCE

UNITED STATES DEPARTMENT OF AGRICULTURE
Agricultural Research Service
Western Utilization Research and Development Division

PROGRAM AND ABSTRACTS OF PAPERS

CITRUS RESEARCH CONFERENCE

January 28, 1958

Fruit and Vegetable Chemistry Laboratory
263 South Chester Avenue
Pasadena, California

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FOREWORD

This Citrus Research Conference is being held to bring to members of the citrus and allied industries in California and Arizona the latest results of research on the chemistry and technology of citrus fruits and fruit products carried on by laboratories of the Agricultural Research Service, U. S. Department of Agriculture. The following Divisions are participating in this year's Conference:

Western Utilization Research and Development Division
Western Regional Research Laboratory (Branch
headquarters), Albany, California
Fruit and Vegetable Chemistry Laboratory, Pasadena, Calif.

Southern Utilization Research and Development Division
U. S. Citrus Products Station, Winter Haven, Florida
U. S. Fruit and Vegetable Products Laboratory, Weslaco, Texas

Tuesday, January 28, 1958

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LUNCH

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HOW PECTINESTERASE EXISTS IN CITRUS FRUIT AND ITS ACTION in situ

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The fact that pectinesterase (PE) does not occur in the juice per se but rather is bound to the insoluble fraction of the fruit (e. g. , "rag") has long been recognized. For this reason PE, as opposed to citrus enzymes which occur naturally in solution within the fruit, can be heat-inactivated only with difficulty. Accordingly a study was made of the manner in which PE is bound to citrus rag as well as the cell walls of other plant tissue.

The "rag" or cell-wall material used was prepared from Valencia oranges by collecting the section membranes and juice-sac walls, suspending in water, homogenizing in a colloid mill followed by exhaustive water washing of the resulting cell walls, and finally suspending them in water. This cell-wall preparation possessed an extremely high PE content.

At the natural pH (3.5) of the cell-wall preparation, salt solutions failed to extract any of the enzyme. However at pH 7.5 and 0.15 N NaCl, deesterification of the in situ pectin and solubilization of the PE proceeded at similar rates, and temperature affected both rates to the same extent, thus suggesting that the deesterification precedes the solubilization of the enzyme. After deesterification was complete, an equilibrium of the enzyme between the cell walls and the extraction medium occurred, which under the conditions used resulted in 50% of the enzyme in solution and 50% in the cell walls. The same equilibrium resulted on subsequent re-extractions, thus demonstrating that even after deesterification of the in situ pectin the cell-wall material has a strong affinity for the enzyme.

As a matter of fact the original cell-wall preparation was found to firmly bind added citrus PE to an extent ~~40~~ times greater than is naturally present. The same was true for cell-wall preparations which had been freed of PE by repeated extractions followed by adjustment of pH of suspensions to 3.5. The affinity of PE for the pectic material in the cell walls was approximately 50 times greater than that for soluble pectin. However, the removal of most of the pectic material from such a suspension through the use of purified polygalacturonase resulted in a loss in ability of cell walls to bind PE.

The determination of the pH-activity relationship of the in situ action of the cell-wall PE as compared with soluble PE demonstrated that both possess the same optimum but that the in situ enzyme was inactive at pH 4.5 and below, whereas the soluble enzyme was still active.

Soluble pectin in relatively large amounts but only in the presence of salt solubilized part of the PE in the cell-wall preparation. In the presence of

0.15 N NaCl and soluble pectin in an amount 10 times that in the cell wall preparation, 8% of the enzyme was solubilized at pH 3.8. The solubilized PE acted on the solubilizing pectin at this pH and rapidly formed a firm gel. Similar results were obtained with cell wall preparation from *avena* coleoptiles (oat seedlings) with respect to the liberation, binding, and in situ action of PE.

Fumarate was found to cause some inhibition of the action of PE in vitro, particularly when rapid-set pectin was used as a substrate. Fumarate and calcium also inhibited the action of PE with slow-set pectin.

The practical implications of these findings as related to juice concentrates will be discussed.

COMPOSITION OF CITRUS CONCENTRATES IN RELATION TO REFRACTOMETER CORRECTIONS FOR ESTIMATING SOLUBLE SOLIDS

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Information was presented last February on effects of various major constituents of citrus on the refractive index of juice concentrates. Since then a series of 54 concentrated citrus juices have been analyzed for citric acid, soluble nitrogen, ash, potassium, glucose, fructose, sucrose, and insoluble solids.

Corrections required for amino acids were found to be relatively large as compared to other constituents; therefore an estimate of the amino acid content of Florida concentrates was essential. For this study it was considered adequate to determine the soluble nitrogen content of the samples, convert that to "mixed" amino acid by use of the factor 5.8, and assume that the nitrogenous materials thus estimated would approximate the effect of refractometer readings of an equivalent solution of mixed amino acids. The range of "mixed" amino acid contents, expressed as percentage of soluble solids, was found to be 3.2 to 5.0, as compared to 3.9% for a single sample of California blood orange, and 6.7% for a single sample of California Valencia concentrate. For juice at a concentration of 40% soluble solids, the correction required for the sample containing 3.2% amino acid would be -0.360° Brix, and for 5.0% the correction would be -0.555° Brix.

The mineral constituents of citrus are known to exist principally in the form of metallic citrates. In order to arrive at an estimate of the mineral content of the series of samples, it was necessary to determine the ash and potassium content of the clarified juices. The range of ash content, expressed as percentage of solids, was found to be 2.80 to 4.30, and the range of potassium citrate 4.90 to 7.65%. Grapefruit juices were slightly lower than orange in mineral content. Corrections required for these amounts of citrates, in 40% concentrates, range from -0.225° to -0.302° .

The reducing sugar content of concentrates from early oranges was found to average 46.6% of total sugars, from midseason oranges 47.9%, and from late oranges 48.7%. Of the reducing sugars, approximately 49% was glucose throughout the season. Unsweetened grapefruit concentrate averaged 59% reducing sugars, of which 57% was glucose. Corrections for glucose in 42° Brix concentrated orange juices averaged $+0.036^{\circ}$, and for fructose $+0.090^{\circ}$. Corrections for reducing sugars in the other citrus concentrates varied only slightly from those required for orange concentrates.

Insoluble solids were determined by a rapid method which resulted in the inclusion of pectinous materials insoluble in alcohol and acetone. For purposes of this study it was assumed that the water-insoluble pulp amounted to 56% of the alcohol-acetone insolubles. The influence of pulp on the refractive index of sugar sirups or fruit juices has not been accurately determined, since pulp which has been frozen or dried and pulp from early-season and from late-season fruit all have effects different from pulp recovered from sound, fresh juice. There is adequate evidence at hand, however, to justify the preparation of a rough curve for estimating corrections for concentrates of varying pulp content when prepared from normal fruit. Pulp content of concentrated orange juice samples was found to vary from 0.564 to 1.057% of the total soluble solids. Tentative correction factors for these amounts of pulp in 42° Brix concentrates are -0.090° and -0.279°, respectively.

Corrections for citric acid, fructose, and glucose are to be added to refractometer readings, while corrections for amino acids, mineral constituents, and pulp are to be subtracted. The net corrections for all orange concentrates studied were found to be negative. After application of the negative corrections, results with these samples indicate that there must still be some unrecognized factors affecting refractometer readings, as the corrected readings were nearly always higher than soluble solids as found by vacuum drying. Uncorrected refractometer readings were usually very close to, but higher than, total solids by drying, while corrected readings were usually closer to total solids than to soluble solids.

RECENT DEVELOPMENTS IN THE CHEMISTRY OF CITRUS FLAVONOIDS

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Flavonoids constitute one of the major groups of compounds in citrus fruits, and are present in considerable numbers and variety. It is altogether likely that certain problems such as darkening, bitterness, and off-flavors of various citrus products are associated with the presence and metabolism of various flavonoids. A more thorough knowledge of these substances will undoubtedly be of value in solving certain of the processing problems, as well as in utilizing flavonoids as valuable intermediates and byproducts. In addition, unanswered questions regarding the role of citrus flavonoids as dietary factors can best be approached by studies of individual pure compounds isolated from citrus. In previous reports the occurrence of diosmin, eriodictyol, and limocitrin in lemons was described. During the past year other flavonoids have been isolated, the identification of which will be described.

The study of citrus flavonoids and flavonoid glycosides is made difficult by the complexity of the mixtures and by the fact that usually only very small amounts of pure materials can be isolated. The elucidation of flavonoid structures can be greatly facilitated by the use of several spectroscopic methods recently developed by workers in this laboratory. These methods are attractive because of their speed, simplicity, and economy of material. The application of these procedures makes it possible to detect spectroscopically in flavonols (1) a free 7-hydroxy group, (2) a free 3, 4'-dihydroxy group, and (3) an ortho-dihydroxy group. Thus, a free 7-hydroxy group is shown by a shift of the short wavelength band to longer wavelengths in the presence of fused sodium acetate. A free 3, 4'-dihydroxy group is shown by the disappearance of the long wavelength band in the presence of sodium ethylate. An ortho-dihydroxy group is indicated by a bathochromic shift in the presence of boric acid-sodium acetate. Use of these procedures enables one to approach a virtually complete structural analysis for several widely occurring types of flavonols. Furthermore, the troublesome problem of the location of the sugar residue in glycosides may often be resolved by comparison of the spectra before and after hydrolysis. Similar spectroscopic studies on flavonones have shown that free 5 and 7 hydroxy groups may be readily detected by various shifts. The usefulness of the above methods will be illustrated by structural problems encountered with limocitrin, neohesperidin, and other citrus flavonoids.

METABOLIC FATE OF HESPERIDIN AND NARINGIN

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At the 1956 and 1957 Citrus Conferences reports were given on the metabolic fate of a number of citrus flavonoids. In summary, these reports showed the citrus flavonoids which are flavanones or flavones undergo a splitting of the molecule in the animal body to yield phenylpropionic acid derivatives. In contrast flavonols such as rutin and quercetin are degraded to yield phenylacetic-acid derivatives. It was shown that the citrus flavonoids, hesperidin, diosmin, and naringin, are poorly absorbed after oral administration as compared with their respective aglycones.

The influence of diet on the absorption of hesperidin is especially noteworthy. On a commonly used commercial diet neither rabbits nor rats excreted the flavonoid or its metabolic products in the urine. On a synthetic diet rabbits receiving hesperidin excreted hesperetin and its glucuronide, 2,4-dihydroxyphenylpropionic acid, 3-methoxy,4-hydroxyphenylpropionic acid, m-coumaric acid, m-hydroxyphenylpropionic acid, m-hydroxyhippuric acid, m-hydroxybenzoic acid, and 3-methoxy,4-hydroxybenzoic acid. On a synthetic diet rats given hesperidin excreted hesperetin and its conjugate, m-hydroxyphenylpropionic acid, and m-coumaric acid. A human given hesperidin on a diet free of citrus fruit and coffee excreted principally 3-hydroxy,4-methoxyphenylhydracrylic acid.

Rats given naringin with a purified diet excreted naringenin and its glucuronide, and para-hydroxyphenylpropionic acid, whereas a human excreted only naringenin and its glucuronide.

COLOR IMPROVEMENT OF CANNED RUBY RED GRAPEFRUIT JUICE

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An increasing amount of Ruby Red and pink-fleshed grapefruit will become available for processing as orchards planted in the lower Rio Grande Valley of Texas come into bearing. Because of the lycopene and carotene pigments in this fruit, special processing techniques are advisable for the production of best-quality canned grapefruit juice. Fortification of the juice with a maximum of pigment-containing pulp intensifies the color, adds provitamin A, and prevents dullness or muddiness from developing in the canned product. Color of the fruit pulp varies from a medium red in the early immature fruit to a light salmon pink in late-season over-mature fruit, and can be correlated with the ratio of lycopene over twice the carotene content, or with the a/b ratio as determined with a Hunter Automatic Color Difference Meter.

In conventional canning methods much of the color is lost when pulp or suspended solids are removed by finishing. This pulp may be recovered by refinishing solids discarded from the first finisher, using brushes instead of paddles. Color-rich pulp may be preserved by canning or freezing for later use in juice when natural fruit color has faded. Limitations on use of pulp are amount of added bitterness and present standards of identity. Current studies indicate enzymatic removal of bitterness of pulp may be a practical method of controlling bitter taste.

SOME CHEMICAL CHANGES ASSOCIATED WITH THE MATURATION OF VALENCIA ORANGES

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The quality of processed orange juice is dependent upon the character of the whole fruit in respect to both physical condition and chemical composition. Physical condition is dependent upon extent of mechanical damage, as well as stage of physiological maturity at the time damage is inflicted. Physiological maturation of oranges is associated with complex chemical changes within the fruit during growth and development. However, economic maturity of oranges is commonly assessed in terms of flavor quality as reflected by the Brix-acid ratio index. Although the latter index has served a very useful function it is generally recognized that it does not always indicate the true organoleptic quality of the juice. Therefore, a systematic study was conducted several years ago to determine the magnitude of some other chemical changes which occur during Valencia orange development in order to obtain a better understanding of the chemical changes which occur during maturation and which might suggest a more satisfactory method for appraisal of fruit maturity or quality.

Quantitative changes have been measured for 12 juice constituents in each of 5 groves of Valencia oranges during the 4-month period (March through June) that rapid development occurs. Factors followed included sugar, total acid, ascorbic acid, alanine, serine, glutamic acid, aspartic acid, γ -aminobutyric acid, proline, arginine, amino nitrogen, total nitrogen, and juice yield.

From data representing the average of 4 groves, a number of interesting correlations were observed among the chemical constituents in relation to development of the fruit. Data presented by other workers for both California and Florida oranges, as well as data reported for other fruits, appear to corroborate the present findings. During the active development period prior and subsequent to maturation, marked changes were detected in rates of accumulation or removal of individual constituents. In other fruits, such as apple, similar changes are associated with the climacteric rise in respiration which in turn is associated with ripening.

One feature common to most fruits exhibiting a climacteric is that they contain some reserve substances such as starch or lipid and that they may ripen after being harvested horticulturally mature. In contrast, citrus fruits have no apparent energy reserve and do not undergo post-harvest ripening in respect to deposition of sugar. Results of the present studies suggest that a climacteric rise in rate of orange respiration may occur on the tree, and that a more reliable estimate of the physiological maturity of the fruit may be obtained by measurements of constituents other than, or in addition to, sugar and acid.

A SPECIFIC QUANTITATIVE COLORIMETRIC METHOD OF ANALYSIS FOR CITRAL IN LEMON OIL

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A modification of the method of Levine and Taterka (Anal. Chim. Acta, 15, 237, 1956) using vanillin-potassium hydroxide reagent for the qualitative detection of saturated aldehydes and ketones has yielded a quantitative colorimetric method of analysis specific for the aldehyde responsible for the characteristic flavor and aroma of lemon oil, i. e., citral. To date, only two related terpene carbonyls have been found to interfere, dihydrocitral and 4-ionone. The reaction is carried out at room temperature in commercial-grade absolute ethanol or methanol with alcoholic vanillin and piperidine reagents. Saturated aldehydes give yellow solutions; saturated ketones, yellow; saturated methyl ketones, red; unsaturated aldehydes, red; and citral and related terpene carbonyls, green (max. absorption 625 m μ).

Of the phenolic aldehydes and phenols tested, only protocatechuic aldehyde can be substituted for vanillin, and of the organic bases only 1, 2, 3, 4-tetrahydroisoquinoline can be substituted for piperidine. The common contaminants in synthetic vanillin, e. g., vanillic acid, iso-vanillin, and guaiacol, do not develop the green color with citral and piperidine. Plots of absorption intensity at 625 m μ against concentration of citral follow Beer's law.

Procedures are described for applying the method to the analysis of citrus oils.

COMPOSITIONAL STUDIES OF CITRUS OILS MAY LEAD TO IMPROVED QUALITY

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Isolation and identification of the soluble solid constituents in commercial lemon oils has been continued and extended to an examination of other citrus oils for comparative purposes. Ten solid compounds have been isolated from lemon oil, 5 of which have been definitely identified and 5 tentatively identified. In the related study of other citrus oils, 10 solid compounds were isolated from lime oil, 4 from grapefruit oil, 5 from Valencia orange oil, 5 from Seville orange oil, and 5 from oil of bergamot. Many of these are new compounds. The knowledge gained concerning differences in distribution of these compounds in different citrus oils has made it possible to develop new and specific methods of analysis for detecting blends of other citrus oils in lemon oil. The presence of 1% grapefruit oil in lemon oil can be detected, for example, by a new method of analysis based on measurement of fluorescence under ultraviolet light.

A new specific colorimetric method of analysis for citral in lemon oil has been used in studying factors affecting citral concentration of lemon oil. The method has also been applied to the study of oil in the oil glands in the flavedo of the fruit. The variation of citral concentration over the surface of the fruit (the "distribution profile") and the effects of growing area, maturity, location on the tree, and effects of sun and shade have been determined on a limited number of samples. Citral losses during processing have been followed where it was possible to do so.

It is apparent that the major loss of citral occurs during pressing of the peel. In this step the loss may be as high as 50%. Contrary to accepted beliefs, leaching of citral by excessive water washing was shown to be negligible. During centrifuging the loss of citral is also negligible.

Identification of the aldehydes other than citral in lemon oil is in progress. Two of these have been identified as normal octyl and normal nonyl aldehyde, compounds whose presence previously had been inferred but not proved definitely.

TIME-TEMPERATURE TOLERANCE OF FROZEN CONCENTRATED ORANGE JUICE

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Frozen concentrated orange juice is dependent on adequate refrigeration to maintain its high initial quality. Processors who have the greatest appreciation of this fact have little control over the handling and storage of frozen concentrates after they leave their plant. Shippers, warehousers, and retailers bear the greatest responsibility for maintaining adequate refrigeration for this product to guarantee quality stability. However, surveys have shown that frozen concentrates are often handled at temperatures which permit rapid deterioration of quality.

Time-temperature-tolerance studies have been performed on commercial frozen orange concentrates over a period of 5 seasons. Nine lots of both heated and unheated concentrates were subjected to fluctuating schedules simulating time-temperature histories encountered under actual conditions. Samples were removed from each lot at the beginning and end of each temperature fluctuation and analyzed for chemical and physical properties which might reflect quality losses.

Results of these experiments indicated that loss of cloud was the first serious change resulting from exposure of concentrates to elevated temperatures, and this quality loss appeared much sooner than did detectable flavor changes. Even in those concentrates which had been heat-stabilized, cloud damage still occurred before flavor damage. It was noted that while concentrates were stored at a constant temperature of 0°F., they became increasingly sensitive with time to loss of cloud when exposed later to higher temperatures for short intervals. Cloud loss is, therefore, not a function of temperature alone, but also a function of age of sample before thawing.

Flavor of frozen orange concentrate was found to be relatively stable during storage. Perceptible damage to flavor appeared only under the more severe time-temperature histories. Although flavor appears to be quite stable in heated concentrates and relatively stable in unheated ones, the effects of time-temperature histories on flavor are cumulative. Unfavorable temperature histories, which do not in themselves produce flavor changes, may nevertheless increase probability of flavor changes appearing after subsequent temperature abuses. That is, when a sufficient number of temperature anomalies accumulate in a sample of frozen concentrate, a flavor change is eventually produced.

Ascorbic acid was found to be extremely stable throughout all time-temperature histories employed. Changes in cloud and flavor in frozen concentrated orange juice may be initiated at all temperatures above 0°F.

LIST OF CITRUS PUBLICATIONS
January 1, 1957 to December 31, 1957

Western Utilization Research and Development Division
Fruit and Vegetable Chemistry Laboratory
263 South Chester Avenue, Pasadena, California

A MODIFIED METHOD FOR QUANTITATIVE ESTIMATION OF BIPHENYL IN CITRUS FRUITS.

W. L. Stanley, S. H. Vannier, and B. Gentili.
Jour. Assoc. Offic. Agr. Chem. 40(1):282-86, Feb., 1957.

CANNING AND STORAGE EFFECTS. VOLATILE WATER-SOLUBLE AND OIL CONSTITUENTS OF VALENCIA ORANGE JUICE.

J. G. Kirchner and J. M. Miller.
Jour. Agr. and Food Chem. 5(4):283-91, April, 1957.

ANALYSIS OF COUMARIN COMPOUNDS IN CITRUS OILS BY LIQUID SOLID PARTITION.

W. L. Stanley and S. H. Vannier.
Jour. Assoc. Offic. Agr. Chem. 40(2):582-88, May, 1957.

CHEMISTRY OF THE VOLATILE CITRUS FLAVORS.

W. L. Stanley.
In Chemistry of Natural Food Flavors--a Symposium published by Quartermaster Food and Container Institute for the Armed Forces (pages 102-12) May, 1957.

CHEMICAL COMPOSITION OF LEMON OIL. I. ISOLATION OF A SERIES OF SUBSTITUTED COUMARINS.

W. L. Stanley and S. H. Vannier.
Jour. Am. Chem. Soc. 79(13):3488-91, July, 1957.

THE TIME-TEMPERATURE TOLERANCE OF FROZEN FOODS. VII. FROZEN CONCENTRATED ORANGE JUICE.

R. J. McColloch, R. G. Rice, M. B. Bandurski, and B. Gentili.
Food Technol., 11(18):444-49, Aug., 1957.

SEPARATION AND IDENTIFICATION OF SOME TERPENES BY GAS PARTITION CHROMATOGRAPHIC ANALYSIS.

R. A. Bernhard.
Jour. Assoc. Offic. Agr. Chem., 40(3):915-21, Aug., 1957.

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THE CAROTENOIDS OF RUBY RED GRAPEFRUIT.

A. L. Curl and G. F. Bailey.

Food Research 22(1):63-68, Jan.-Feb., 1957.

COMPARISON OF XANTHOPHYLLS FROM LEAVES AND ORANGE JUICE.

A. L. Curl and G. F. Bailey.

Food Research 22(3):323-29, May-June, 1957.

TANGERINE CAROTENOIDS.

A. L. Curl and G. F. Bailey.

Jour. Agr. and Food Chem. 5(8):605-8, Aug., 1957.

Southern Utilization Research and Development Division
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COMPOSITION OF COMMERCIAL, SEGMENT, AND PEEL JUICES OF
FLORIDA ORANGES.

L. J. Swift and M. K. Veldhuis.

Jour. Agr. and Food Chem. 5(1):49-52, 1957.

*STABILITY OF FROZEN CONCENTRATED ORANGE JUICE. III. EFFECT
OF HEAT TREATMENT IN PRODUCTION OF HIGH-BRIX FROZEN CON-
CENTRATE.

O. W. Bissett, M. K. Veldhuis, R. B. Guyer, and W. M. Miller.

Food Technol. 11(2):96-99, 1957.

*----- IV. EFFECT
OF HEAT TREATMENT OF HAMLIN, PINEAPPLE AND VALENCIA JUICES
AT DIFFERENT STAGES OF MATURITY.

O. W. Bissett, M. K. Veldhuis, R. B. Guyer, and W. M. Miller.

Food Technol. 11(10):512-15, 1957.

*----- V. EFFECT OF
HEAT TREATMENT AT INTERMEDIATE STAGES OF CONCENTRATION ON
JUICES PREPARED FROM VALENCIA ORANGES.

E. A. Carroll, R. B. Buyer, O. W. Bissett, and M. K. Veldhuis.

Food Technol. 11(10):516-19, 1957.

